African Journal of Biotechnology Vol. 6 (7), pp. 877-880, 2 April 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Investigation of plasmid DNA and antibiotic resistance in some pathogenic organisms

UCHECHI N. Ekwenye and ERINMA Kazi

Department of biological sciences, Michael Okpara University of Agriculture. P.M.B 7267. Umuahia, Abia State. Nigeria.

Accepted 20 May, 2005

Twenty-eight strains of *Salmonella*, *Pseudomonas*, and *Escherichia coli* isolated from cultures of stool, urine and wound were tested for their susceptibility to various antimicrobial agents. All the strains were resistant to erythromycin and tetracycline. Nineteen *Salmonella* isolates were susceptible to chloramphenicol and gentamycin. All the *Pseudomonas* and *Escherichia coli* isolates were resistant to chloramphenicol and cloxacillin while one *Pseudomonas* species and three *Escherichia coli* isolates were sensitive to gentamycin. The antibiotic resistance determinants in each strain were encoded by plasmid. These isolates were typed by plasmid profile analysis according to their different molecular weights. Several similar and distinct profiles were identified for most resistant and sensitive isolates. It appeared that a single strain containing a plasmid conferring multiple drug resistance emerged within the bacterial population and was able to adapt and to survive the challenges of antibiotics as they were introduced into clinical medicine. Therefore, the acquisition of plasmid has greatly contributed to the rapid spread of antibiotic resistance genes in the bacterial population.

Key Words: Susceptibility, antibiotics, plasmid DNA, drug resistance.

INTRODUCTION

Salmonella, Pseudomonas, and *Escherichia coli* isolates has now been established as etiological agents of human gastroenteritis, enteric fever, septicemia, localized infections and diarrhea disease of humans (Hadfield et al., 1985). These pathogenic organisms also become resistant as a result of genetic mutations or acquisition of pre-existing genes that confer resistance (Summers, 1996), which occur either in the deoxyribonucleic acid (DNA) of the bacteria chromosomes or in the extra chromosomal transferable DNA called plasmids (Brown et al., 1996). Thus antibiotic resistance can be disseminated to other bacteria by the plasmid during conjugation (Karmaker et al., 1991). The rapid spread of antibiotics resistance genes in bacterial population is due to selective pressures resulting from the intensive and the indiscriminate use of antibiotics in human therapy (Couturier et al., 1988).

The association of these pathogenic organisms with diseases in humans has increased the importance of epidemiological studies. Historically, serotyping has been extensively used for identification of *Salmonella, Pseudomonas,* and *E. coli* for epidemiological purposes but several other methods, including phage typing, biotyping, antibiotic resistance determination and plasmid profile analysis, are now available. A number of plasmid screening procedures which vary in subtle ways have been used for the detection of plasmid (Fujita et al., 1994). In this study, the existence of plasmid DNA carrying antibiotic resistance genes in *Salmonella*, *Pseudomonas*, and *E. coli* isolates obtained from stool, urine and wound infections from the Lagos University Teaching Hospital (LUTH) were investigated.

MATERIALS AND METHODS

Bacterial strains

Twenty eight bacterial strains were isolated from stool, urine and wound from clinical patients at Lagos University Teaching Hospital (LUTH), Nigeria. All the strains were grown in stock culture bottles containing Muller-Hinton Agar (MHA) and incubated at 37°C for 24 h. Cultures were stored at 4°C until required (Owen and Hernandez, 1990).

*Corresponding author. E-mail: ekwenyeuchechi@yahoo.com.

Table 1. Antibiotic resistance pattern in *pseudomonas.*

CODE NO.	GEN	COT	CHL	AUG	AMX	ERY	TET	CXC
P29	R	R	R	R	S+	R	R	R
P24	S+	R	R	R	R	R	R	R
P26	R	R	R	R	R	R	R	R
P71	R	R	R	R	R	R	R	S+
P 45	R	R	R	S+	R	R	R	R
P14	R	R	R	S+	R	R	R	R

Key: R – Resistance; S – Sensitive; GEN – Gentamycin; COL – Cotrimoxazole; CHL – Chloramphenicol; AUG – Augmentin; AMX – Amoxicillin; ERY – Erythromycin; TET – Tetracycline; CXC – Cloxacillin.

Table 2. Antibiotic resistance pattern in *salmonella.*

CODE NO.	GEN	COT	CHL	AUG	AMX	ERY	TET	CXC
ER100	S+++	R	S++	R	R	R	R	R
ER17	S+++	R	S++	S++	R	R	R	R
ER23	S+++	R	S++	R	R	R	R	R
ER12	S+++	S+++	S++	R	R	R	R	R
ER46	S++	R	S++	R	R	R	R	R
ER	S++	R	S++	R	R	R	R	R
ER142	S+++	R	S++	S++	R	R	R	R
ER14	R	R	S++	R	R	R	R	R
ER2	S+++	S+++	S+++	S+++	S+++	R	S+	R
ER80	S++	R	S++	R	R	R	R	R
ER4	S+++	R	S++	S+	R	R	R	R
ER121	S+++	R	S++	R	R	R	R	R
ER30	S+++	S+++	S+++	R	R	R	R	R
ER145	S+++	S+++	S++	S+++	S+++	R	R	R
ER51	S++	R	S++	S++	R	R	R	R
AZ	S+++	R	S++	R	R	R	R	R
SE18	R	R	S++	R	R	R	R	R
OD	S+++	S+++	S++	S+++	S+++	R	R	R
AP	S+++	R	S++	S++	S++	R	R	R

Key: R – Resistance; S – Sensitive; GEN – Gentamycin; COL – Cotrimoxazole; CHL – Chloramphenicol; AUG – Augmentin; AMX – Amoxicillin; ERY – Erythromycin; TET – Tetracycline; CXC – Cloxacillin

Susceptibility tests

Isolates were screened for resistance to gentamycin (10 mg/ml), cotrimoxazole (25 mg/ml), chloramphenicol (30 mg/ml), augmentin (30 mg/ml), amoxicillin (25 mg/ml), erythromycin (5 mg/ml), tetracycline (30 mg/ml) and cloxacillin (5 mg/ml), using commercial multiple discs (Habdisc-Gram negative, by ABTEK biological limited Liverpool) stored in 2-8°C. All *Salmonella, Pseudomonas* and *E. coli* isolates wee plated out from the stock culture bottles on the newly prepared Muller Hinton Agar plates. Plates were incubated at 37°C for 24 h. Characterization of strains as sensitive or resistant was based on the size of the inhibition zones around each disc.

Isolation of plasmid DNA

Plasmid DNA was extracted from *Salmonella, Pseudomonas* and *E. coli* isolates by alkaline lysis method (Birnboim and Doly, 1979) and electrophoresed in 0.4% agarose slab in TBE running buffer (540 g Tris, 225 g borate and 41.5 g EDTA dissolved in 500 ml of water at pH 8.0) containing ethidium bromide (1 mg/ml). The gels were photographed under ultra-violet illumination using the Polaroid film. The molecular sizes of each plasmid were determined by comparison with plasmids of known mass (Datta et al., 1971).

RESULTS AND DISCUSSION

All the *Salmonella, Pseudomonas* and *E. coli* isolates were found to be resistant to one or more of the antimicrobial agents tested with eight different patterns of antibiotic resistance. All Pseudomonas isolates displayed resistance towards tetracycline, erythromycin, chloramphenicol and cotrimoxazole (Table 1). All *Salmonella* isolates showed resistance to tetracycline, erythromycin and cotrimoxazole. However, none of the *Salmonella* isolates were resistant to chloramphenicol and Gentamycin (Table 2). The *E. coli* isolate displayed resistance to-

Table 3. Antibiotic resistance pattern in *Escherichia coli.*

CODE NO.	GEN	COT	CHL	AUG	AMX	ERY	TET	CXC
EC51	S+	R	R	S+	R	R	R	R
EC68	S+	R	R	R	S+	R	R	S+
EC45	S+	R	R	R	R	R	R	R

Key: R – Resistance; S – Sensitive; GEN – Gentamycin; COL – Cotrimoxazole; CHL – Chloramphenicol; AUG – Augmentin; AMX – Amoxicillin; ERY – Erythromycin; TET – Tetracycline; CXC – Cloxacillin.

Table 4. Plasmid profiles of the strains harbouring plasmids.

Strain	Code No.	No. of Plasmid	Estimated Plasmid Size (kb)
Salmonella	ER30	1	23.13
	ER46	4	2.09, 2.322, 1.047, 1.035
	ER14	3	2.09, 2.322, 1.1075
	ER121	1	23.13
	SE18	1	23.13
	ER17	1	23.13
Pseudomonas	P29	1	18.03
	P71	1	19.36
	P14	2	4.361, 19.36
	P45	1	18.03
	P26	1	23.13
E. Coli	EC68	1	23.13

Note: ER23, AZ, ER100, ER12, P24, EC52, EC45, NO PLASMID was detected.

Table 5. Molecular weight sizes of the marker.

Molecular sizes of the marker in (kb)	Log of molecular sizes of the marker	Distance moved by the bands in (cm)
23.13	1.36	1.5
9.416	0.97	2.4
6.557	0.82	2.9
4.361	0.64	3.5
2.322	0.37	4.0
2.09	0.32	4.2

wards tetracycline, erythromycin, chloramphenicol and contrimoxazole (Table 3). The results of the plasmid screening were profiled as summarized in Table 4 and 5.

Approximately 63.2% of the isolates harbored plasmid; 47.4% containing one plasmid, one contained two plasmids, one contained three plasmids and one contained four plasmids. The plasmids ranged from 1.00 to 23.13 kb. Plasmid DNA was found in 60, 33.3 and 83.3 of the *Salmonella*, *E. coli* and *Pseudomonas* isolates, respectively. Approximately 50% of these plasmids were 23.13 kb. Four tetracycline and chloramphenicol resistance *Pseudomonas* isolates carried 2.09, 2.322, 1.047 and 1.035 kb plasmids and one had 2.09, 2.322 and 1.175 kb plasmids. Seven isolates apparently had no plasmids. The results showed that all the isolates conferred resistance to one or more of the various antibiotics used. The frequency of antibiotic resistance in *Salmonella* has been found to be low and stable (Cohen and Tauxe, 1986). In contrast, most highly prevalent *Salmonella* isolates harboring plasmid are usually chloramphenicol resistant. All chloramphenicol resistant *Salmonella* strains are plasmid mediated (Mourad et al., 1993. However, resistance to tetracycline and erythromycin appear to be common (Hadfield et al., 1985).

A plasmid occurrence rate of 63.2% was observed between the various isolates, with 25% harboring multiple plasmids. The plasmid ranged form 1.00 to 23.13 kb, with the 23.13 kb being the most common plasmid detected. The high prevalence of the strains carrying a 23.13kb plasmid, singularly or in combination with other plasmids

has been reported previously (Helmuth et al., 1985).

Different plasmids having same or similar weights but expressing different resistance phenotype was observed. Therefore, the inability of these isolates to harbor plasmids does not affect its ability to confer resistance to the various antibiotics.

ACKNOWLEDGEMENT

The authors are grateful to the Microbial Genetics and Biotechnology staff of the Nigerian Institute of Medical Research, Lagos.

REFERENCES

Birmboim HC, Doly J (1979). A rapid alkaline extraction procedure for screening recombinant DNA. Nucleic Acids Res. 7:1513-1523.

Brown JC, Shanahan PMA, Jesudason MV, Thomas CJ, Amyes SG. (1996). Mutation responsible for reduced susceptibility to 4-quinolones in clinical isolates of multi-resistant *Salmonella typhi* in India. J. Antimicrobial Chemotherapy 37:891-900.

Cohen ML, Rauxe RV (1986). Drug resistant *Salmonella* in the United State: an epidemiologic perspective. Science 234:946-969.

Couturier M, Bex F, Berquist PL, Maas WK (1988). Identification and Classification of bacterial plasmids. Microbial Rev. 52: 375-395.

Datta N, Hadges RW, Shaw EJ, Sykes RB, Richman MH (1971). Properties of an R factor from *Pseudomonas aeruginosa*. J. Bacteriol. 108:1244-1249.

Fujita M, Ike M (1994). Waste waters treatment using Genetically Engineered Microorganisms. Basel: Technomic publishing company.

Hadfield TL, Monson MH, Wachsmuth IK (1985). An outbreak of antibiotic-resistant *Salmonella Enteritidis* in Liberia, West Africa. J. infectious Dis. 151:790-795.

Helmuth R, Stephen R, Bungo C, Hoog B, Steinbeck A, Bulling E (1985). Epidemiology of Vimlence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. Infection and community 48: 175-182.

Karmaker S, Biswas D, Shaikh NM, Chatterjee SK, Kataria VK, Kumar R (1991). Role of large plasmid of *Salmonella typhi* encoding multiple drug resistance. J. Med. Microbiol. 34: 149-151.

Mourad AA, Metwally M, El Deen AN, Threlfall EJ, Rowe B, Mapes T, Hedstrom R, Bourgeois AL, Murphy JR (1993). Multiple drug resistant *Salmonella typhi.* Clin. infectious Dis. 17:135-136.

Owen RJ, Hernandez J (1990). Occurences of plasmids in camphylobacter upsalensis (Catalase negative or weak group) from geographically diverse patients with gastroenteritis or bacteremia Eur. J. Epidemiol. 6:11 1-117.

Summers DK (1996). The biology of plasmids. Cambridge: Mass Blackwell sciences Ltd.